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Research Article

A New Calcium Source for Bioactive Sol-Gel Hybrids

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Abstract Poly(γ-glutamic acid) (γ-PGA) has been introduced into sol-gel derived silica to produce an organic/inorganic hybrid system to improve the toughness of conventional bioactive glass. Since calcium is an important element in bioactive glass for bone regeneration, this work is trying to find a suitable calcium source for this hybrid system and determine how to incorporate the calcium within the γ-PGA hybrid. Calcium chloride and calcium methoxyethoxide (CME) were tested as calcium sources in this work. CME was found to incorporate into the silica network better than calcium chloride at low temperature.

Keywords calcium methoxyethoxide; poly(γ-glutamic acid); hybrid; sol-gel; MAS-NMR

1 Introduction

Bioactive glasses for bone regeneration have been produced that are osteogenic and resorbable [2]. However, they are brittle. Incorporation of biodegradable polymer into the inorganic glasses is an excellent way of toughening the system. Recently, a novel organic/inorganic hybrid system has been developed by our group. This hybrid is produced in a one pot synthesis where the bioactive silica sol and the organic poly-γ-glutamic acid (γ-PGA) are mixed together and allowed to integrate. The hybrid system was also a class II system where the polymer and silica are linked together by covalent bonding using the organosilane glycidoxypropyltrimethoxy silane (GPTMS), this importantly allows for controlled dissolution of the hybrid material.

Calcium is very important in the system as it plays an extremely important role in the formation of hydroxyl carbonate apatite (HCA) layer which provides bonding with the bone. It is also thought to act as a signalling agent to osteogenic cells as it is released from bioactive glass [1]. However, traditional sol-gel calcium sources are not suitable for low temperature hybrid materials. Calcium nitrate is the traditional calcium source for bioactive sol-gel glasses it however requires to be heated over 400 °C to remove the toxic nitrate by-products [3]. This heat treatment is not suitable for the polymer as it will be burned off. A new calcium source is therefore required which does not release toxic by-products and is incorporated into the hybrid system at low temperatures (e.g. lower than 60 °C). One such calcium source is calcium methoxyethoxide (CME). In this study the properties of the CME hybrid samples have been compared with samples made with another potential calcium source calcium chloride. Traditional sol-gel derived bioactive glasses made with CME have been reported to form a thicker and more compact apatite layer than conventional bioactive glasses [4].

2 Materials and methods

Calcium methoxyethoxide (CME) is an alkoxide which was synthesised by reacting calcium metal with 2-methoxyethanol under argon atmosphere at 80 °C for 24 h. The ratio of the reaction was 1 g calcium metal to 24 ml 2-methoxyethanol. The resultant solution was centrifuged to remove unreacted calcium metal and the CME solution was obtained with a concentration of 0.001 mol/ml.

Class I and Class II hybrid samples were made, where in the former only weak Van der Waal's forces, hydrogen bonding and/or ionic bonding exists and in the latter strong covalent bonding was present due to the use of GPTMS. Figure 2 shows a flow chart of the different synthesis methods used. The polymer had to be dissolved in dimethyl sulfoxide (DMSO) to create a polymer solution. Class I and Class II hybrid were made with $CaCl_2$ and CME. Class I hybrids were made by mixing the organic polymer solution and the inorganic bioactive silica sol together. The Class II hybrids were synthesised by firstly functionalising the γ-PGA by reacting it with GPTMS in DMSO under argon atmosphere at 80 °C as shown in Figure 1. The molar ratio of γ-PGA to GPTMS was 2:1 and the reaction was carried out for 12 h.

For calcium chloride samples the silica sol was prepared by adding $CaCl_2$ and mixing for 30 minutes and large amounts of DMSO were removed by rotary vacuum evaporator before the polymer solution was added. Then

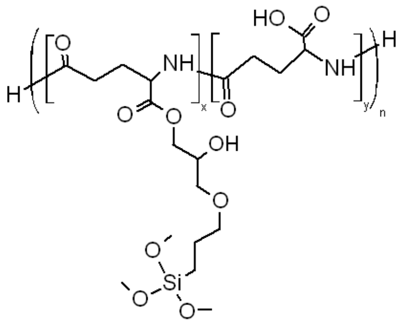

Figure 1: Poly(γ-glutamic acid) functionalised with the organosilane glycidoxypropyltrimethoxy silane.

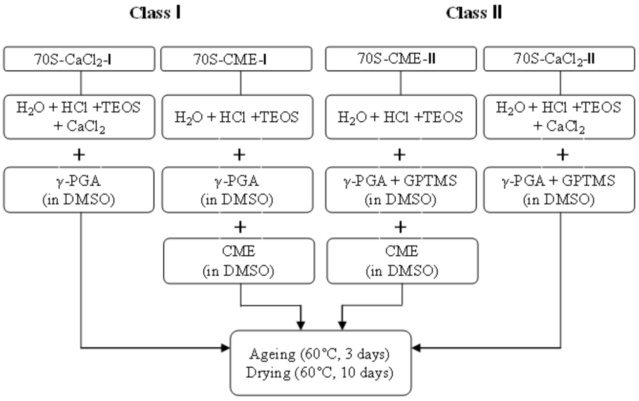

Figure 2: Flow chart of synthesis of Class I and Class II γ-PGA/bioactive silica hybrids.

the sol was mixed with the functionalised γ-PGA solution for 1 h in Teflon moulds at room temperature. Some of the hybrids were gelled with hydrofluoric acid (HF) with a ratio of 3 ml HF to 50 ml solution. For CME samples, TEOS was initially hydrolyzed for 30 minutes by water using R = 2 (R is the molar ratio of H_2O to TEOS). These two systems were then mixed together at room temperature in Teflon moulds then CME was added in slowly while continuously mixing. DMSO was not evaporated to assist with the homogeneous dispersion of CME in the mixture. The mixture gelled in 5 minutes, no HF was added. The weight percent of organic/inorganic was 40/60 (40P) or 60/40 (60P) while the molar ratio of Si/Ca was 70/30 to make the inorganic the same composition as the 70S30C (70 mol% SiO_2, 30 mol% CaO) bioactive glasses. The Teflon moulds were placed into oven directly then aged at 60 °C for 72 h and dried at 60 °C for 10 days.

Network connectivity of the hybrid samples was tested by ^{29}Si NMR. Ca incorporation into the silica network was assessed by ^{29}Si solid state NMR. The mesoporosity was quantified by nitrogen sorption and dissolution of the hybrids in simulated body fluid (SBF) was also tested.

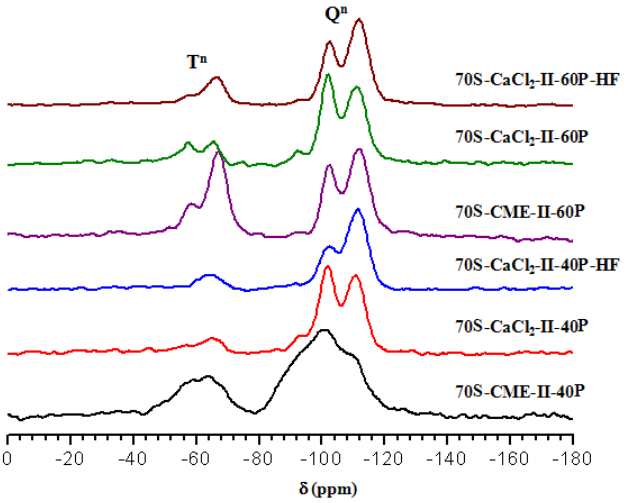

Figure 3: ^{29}Si NMR spectra of hybrids with different Ca sources.

3 Results and discussion

All hybrid samples were successfully produced with $CaCl_2$ and CME as a calcium precursor. The CME alkoxide was highly reactive in comparison with silica precursor TEOS, therefore as soon as it was in contact with water it would form a gel. To overcome this additional DMSO was added to slow this reaction down. In dissolution tests in simulated body fluid (SBF), all Class I hybrid samples dissociated within 15 seconds, whereas Class II samples remained intact for longer. This is because the coupling agent GPTMS provides covalent bonding between the polymer and silica to enhance the connectivity of hybrid. The GPTMS also improves the integration of the organic and inorganic systems. 70S-$CaCl_2$-II samples remained intact in SBF for over a month, whereas 70S-CME-II samples started to break up after 4 h. The 60 wt% 70S-CME-II samples were found to be more stable than the 40 wt% 70S-CME-II samples. The lower stability of the 70S-CME-II samples were due a combination of two reasons: (1) the presence of large amounts of DMSO; and (2) the accelerated gelling of the solution by the CME. Both occurrences reduced the time for silica network formation. The silica network may have only formed partially and not throughout the whole sample. 70S-CME-II that were gelled with HF remained intact in SBF for up to 4 days compared to 4 h without HF, which is due to the HF catalysing the gelling of the silica network.

The network connectivity of different species was obtained by the analysis of ^{29}Si solid state NMR spectra (Figure 3) and summarised in Table 1. Here Q^n indicates the number of bridging O connecting to Si where maximum number of bridging oxygen is 4 and T^n indicates the number of bridging oxygen bonds connecting to a Si that is connected to one C (i.e. the polymer). From the table,

Sample	T^1		T^2		T^3		Q^2		Q^3		Q^4	
	δ (ppm)	I (%)	δ (ppm)	I (%)	δ (ppm)	I (%)	δ (ppm)	I (%)	δ (ppm)	I (%)	δ (ppm)	I (%)
70S-CME-II-40P	–49.6	4	–57.1	8	–65.0	14	–91.3	22	–100.9	32	–110.7	20
70S-$CaCl_2$-II-40P	—	—	–56.2	3	–64.8	8	–92.9	7	–101.5	40	–110.7	42
70S-$CaCl_2$-II-40P-HF	—	—	–60.0	3	–65.1	9	–91.2	3	–102.0	28	–111.4	58
70S-CME-II-60P	–50.8	1	–58.2	11	–66.9	30	–92.2	1	–102.2	20	–111.6	37
70S-$CaCl_2$-II-60P	–52.4	2	–57.4	8	–65.1	9	–92.7	5	–101.8	33	–110.9	43
70S-$CaCl_2$-II-60P-HF	—	—	–57.8	6	–66.2	14	–93.5	2	–102.3	27	–111.6	51

Table 1: Results of the analysis of the ^{29}Si MAS-NMR spectra. 70S signifies the 70S30C composition of the inorganic; CME or $CaCl_2$ indicate the calcium source used; II shows a class II hybrid (GPTMS used as coupling agent); 40P and 60P indicate the wt% organic; HF signifies use of HF where appropriate.

comparing 40 wt% polymer samples, 70S-CME-II-40P had higher T^3 content than 70S-$CaCl_2$-II-40P and 70S-$CaCl_2$-II-40P-HF, but lower Q^4, indicating a high degree of coupling between the silica and the polymer. The 70S-CME-II-40P sample showed much higher percentage of Q^2 than the samples synthesised with $CaCl_2$, indicating improved calcium incorporation into the silica network at low temperature using CME. However, this did not occur when the percentage organic was increased (70S-CME-II-40P). Similar proportions of T and Q groups were found for the 60 wt% polymer samples. However, since in these samples the higher wt% phase was the polymer, the higher condensed T network helped stabilise the hybrid. All hybrids containing calcium chloride had higher percentage of Q^4 groups, indicating that the silica network was very well formed and that the Ca was not incorporated into the network. Adding of HF into the calcium chloride samples increased the number of Q^4 groups, increasing the network connectivity and stability in SBF, but did not affected the T species, indicating that the HF was only involved in the condensation of the hydrolysed TEOS.

All the hybrid samples had no mesoporosity as determined according to nitrogen sorption. This was because the polymer was found to fill the mesopores within the silica network.

4 Conclusions

Hybrid materials were successfully produced using $CaCl_2$ and CME as the Ca precursors. The CME was found to induce gelling on mixing with the organic/inorganic mixture. This was beneficial as rapid gelation is needed to produce porous foam scaffolds, which is usually achieved using HF as a catalyst. Therefore CME eliminates the need to use HF as a gelling agent. ^{29}Si NMR showed T-species in the hybrids and the T-species content increased with increasing polymer content. The amount of polymer and the calcium source were found to greatly influence the dissolution of the hybrids.

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